Supporting Information

for

Fluorogenic Detection of Monoamine Neurotransmitters in Live Cells

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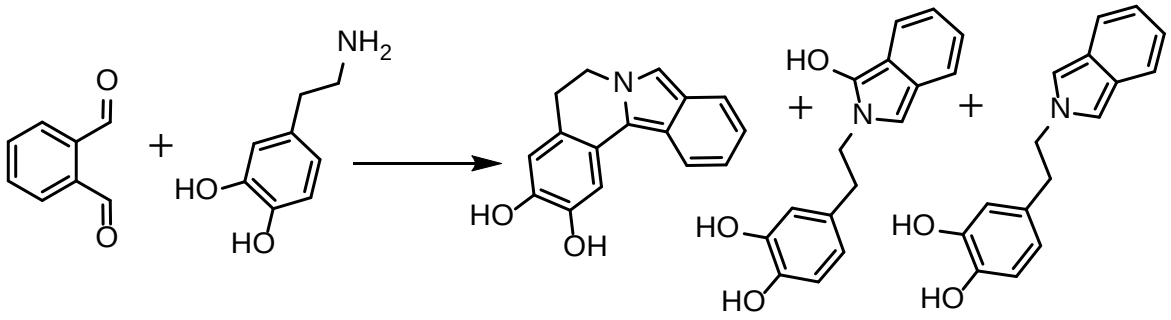
Contents

1. Reaction scheme :Dopamine (DA) and OPA adduct

Equivalent concentration of dopamine and OPA are mixed in 10 mM Thomson's buffer(pH= 7.4) at room temperature (25 ºC) for 30 min in stirring condition.



(I) (II) (III)

m/z_{cal} = 251.09 367.00 253.11

Figure S1: A plausible adducts formation scheme upon reaction with dopamine (DA) and OPA.

2. Mass spectra of the dopamine (DA) OPA adduct

1 mM of OPA (in 500 µL 10 mMThomson's buffer, pH = 7.4) and 1 mM of DA:in 500 µL Thomson's buffer, pH = 7.4) aremixed for 30 minutesthen the reaction mixture was desalted using ziptipand the reaction products were dissolved in acetonitrile:water (1:1). This mixture was used for confirming possible products using MALDI-TOF mass spectrometer (Bruker model ultrafleXtreme) and ESI mass spectroscopic technique.

2.1 MALDI spectra of the dopamine (DA) OPA adduct

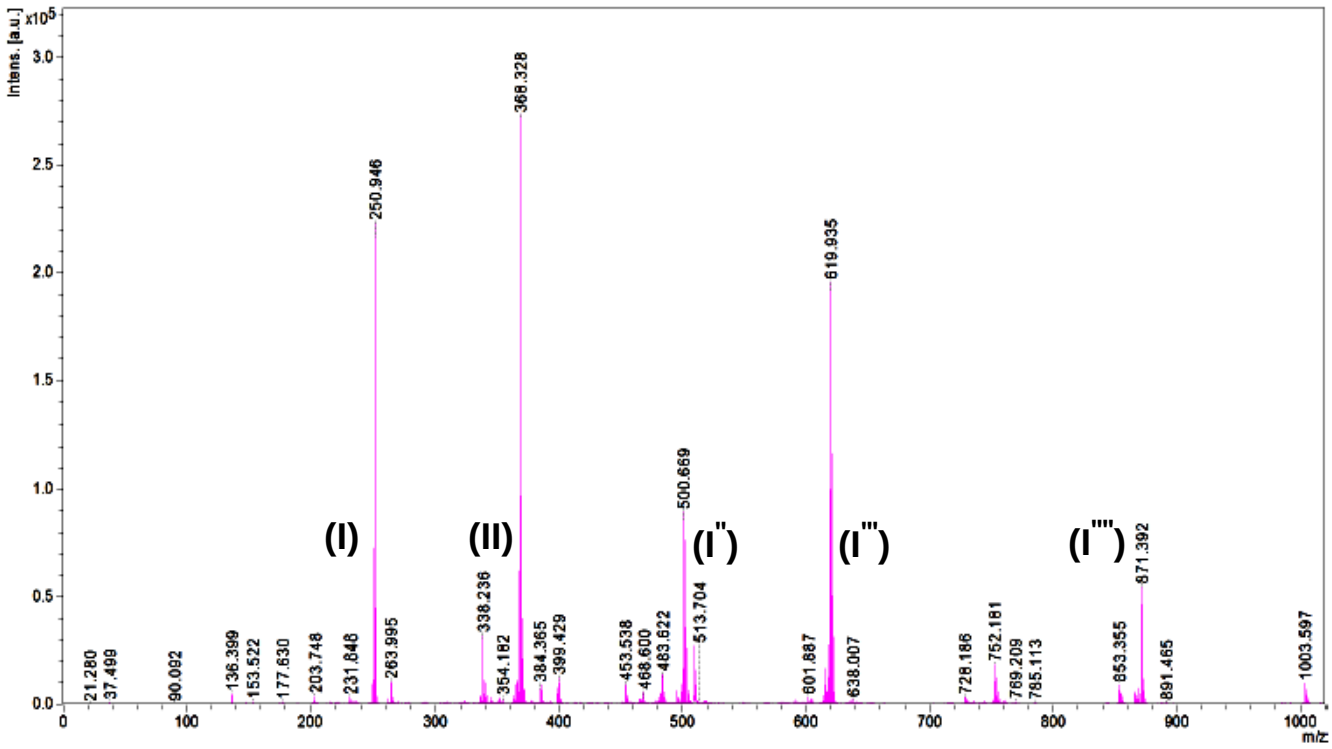


Figure S2: MALDI TOF mass spectra of dopamine-OPA (DA-OPA) adducts.

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m/z= 250.946 indicate formation ofI(m/z_{cal}= 251.09),

m/z= 388.328 indicate formation of II(m/z_{cal}= 367.00)

We didn't get the signature of the product III in the MALDI spectrum. However, we have found higher molecular mass which is possibly due to polymerization of DA-OPA adducts through catechol moiety. These polymerization reactions are described in the next section.

2.1.1. Reaction mechanism for OPA dopamine adducts for product I and II

Product I:

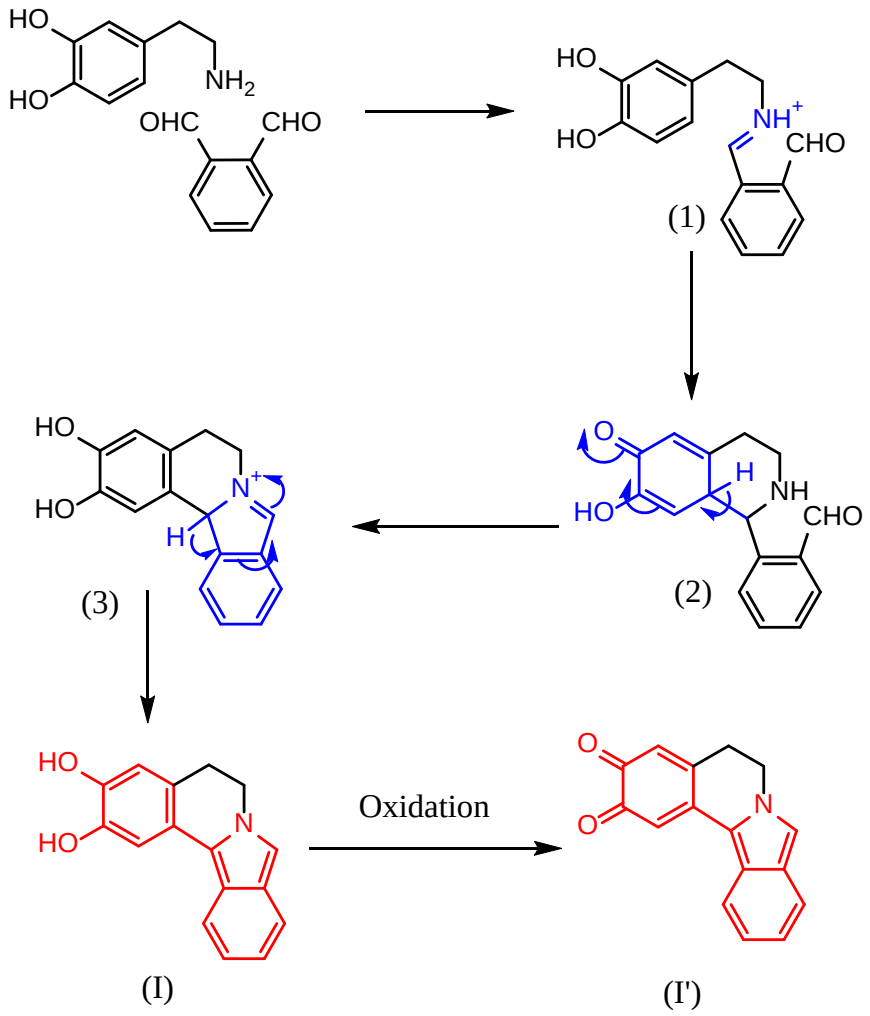


Figure S3: Possible reaction mechanism for the product I

The formation of this product I is already confirmed by MALDI (Figure S2)

Product II:

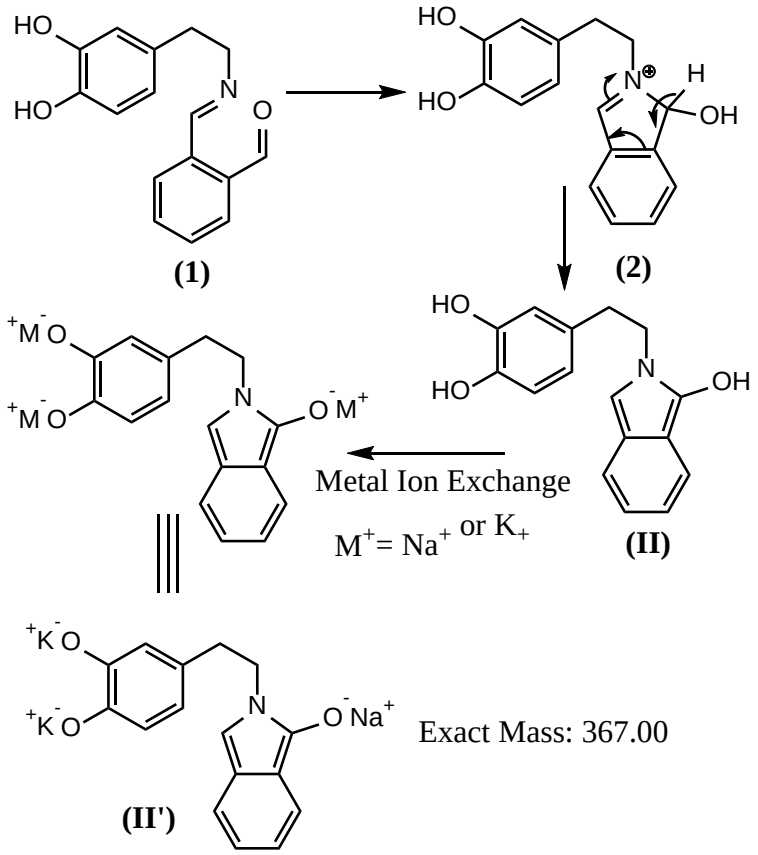
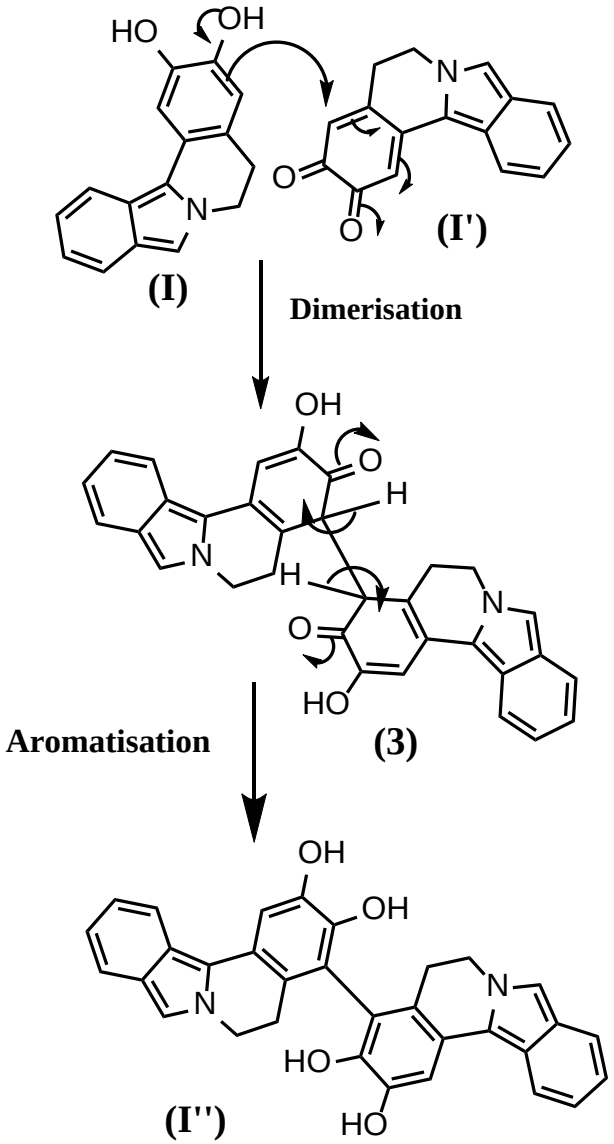


Figure S4: Possible reaction mechanism for the product II

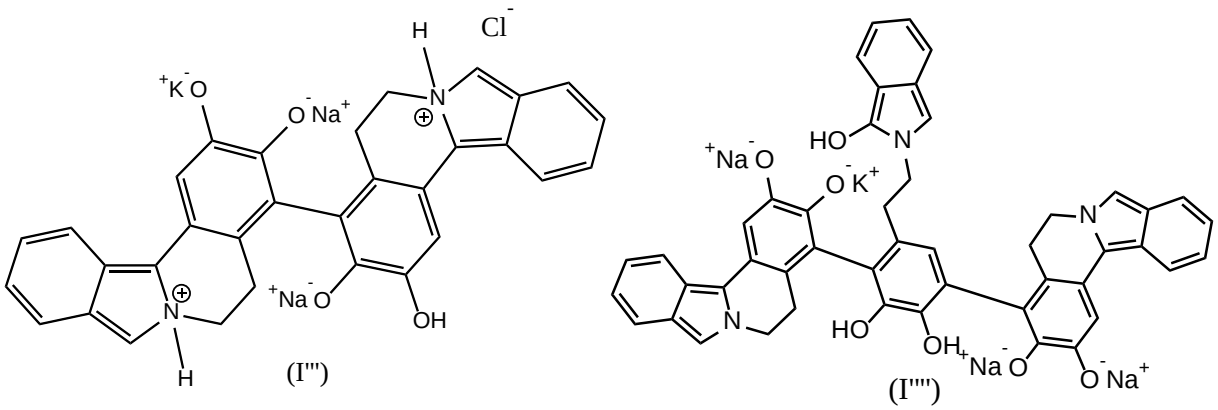
The formation of this product II is confirmed by MALDI (Figure S2)

2.1.2. Plausible reaction mechanism of higher molecular weight adduct formation



Exact Mass: 500.17

Figure S5: Possible reaction mechanism for higher molecular mass



Dimer (I''') Trimer (I'''')

Figure S6: Structure for the dimer and trimer.

m/z =550.669 indicate formation of DA-OPA dimerI" (m/z_{cal}= 550.17)

m/z= 619.935 indicate formation of DA-OPA dimer with Na^+& K^+ salts I''' (m/z_{cal}=619.08)

m/z= 871.392 indicate formation of DA-OPA trimerI'''' (m/z_{cal} = 871.16).

Such polymerization also support formation of I and II.

Note: In the cellular environment, the above polymerization step could not take place as it is reducing in nature.

2.2 ESI-MS spectra of the Dopamine (DA) OPA adduct

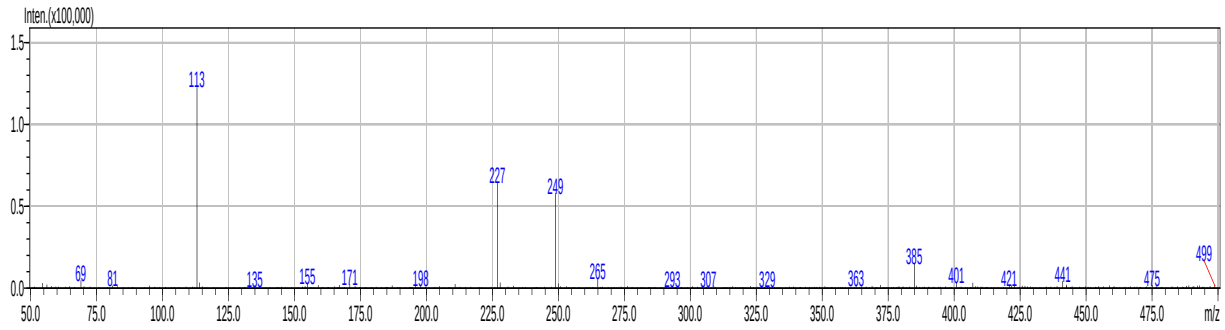


Figure S7: ESI Mass spectra of dopamine-OPA (DA-OPA) adduct.

m/z = 249.0 indicate the formation of I′ (oxidized form of I)

3. OPA-serotonin reaction:

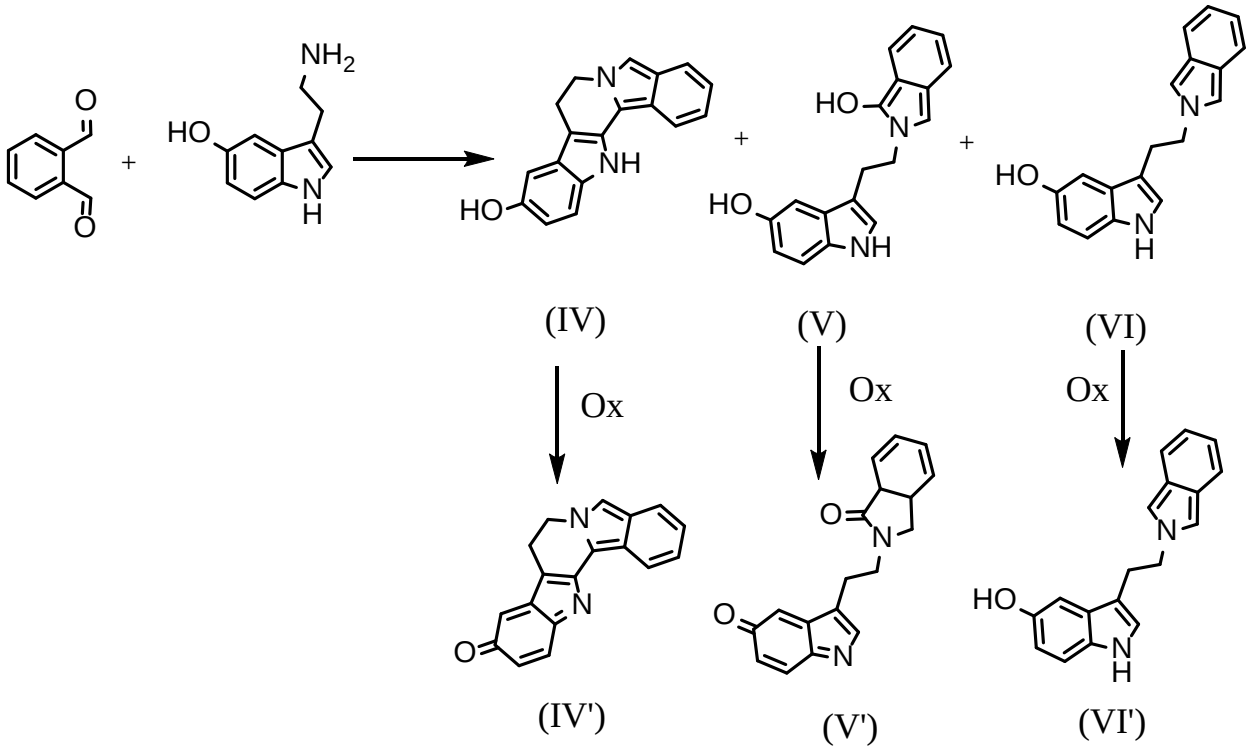


Figure S8: A plausible adducts formation scheme upon reaction with serotonin and OPA.Ox stands for oxidation.

Note: Serotonin and OPA can form adducts by similar kind of reaction mechanism.

4.1 MALDI spectra of the serotonin (5-HT) OPA adduct

1 mM of OPA (in 500 µL 10 mMThomson's buffer, pH = 7.4) and 1 mM of 5-HT(in 500 µL 10 mM Thomson's buffer, pH = 7.4) aremixed for 30 minutes then the mass spectra are checked of the final products after ziptip(C18) using MALDI-TOF mass spectrometer (Bruker model ultrafleXtreme).

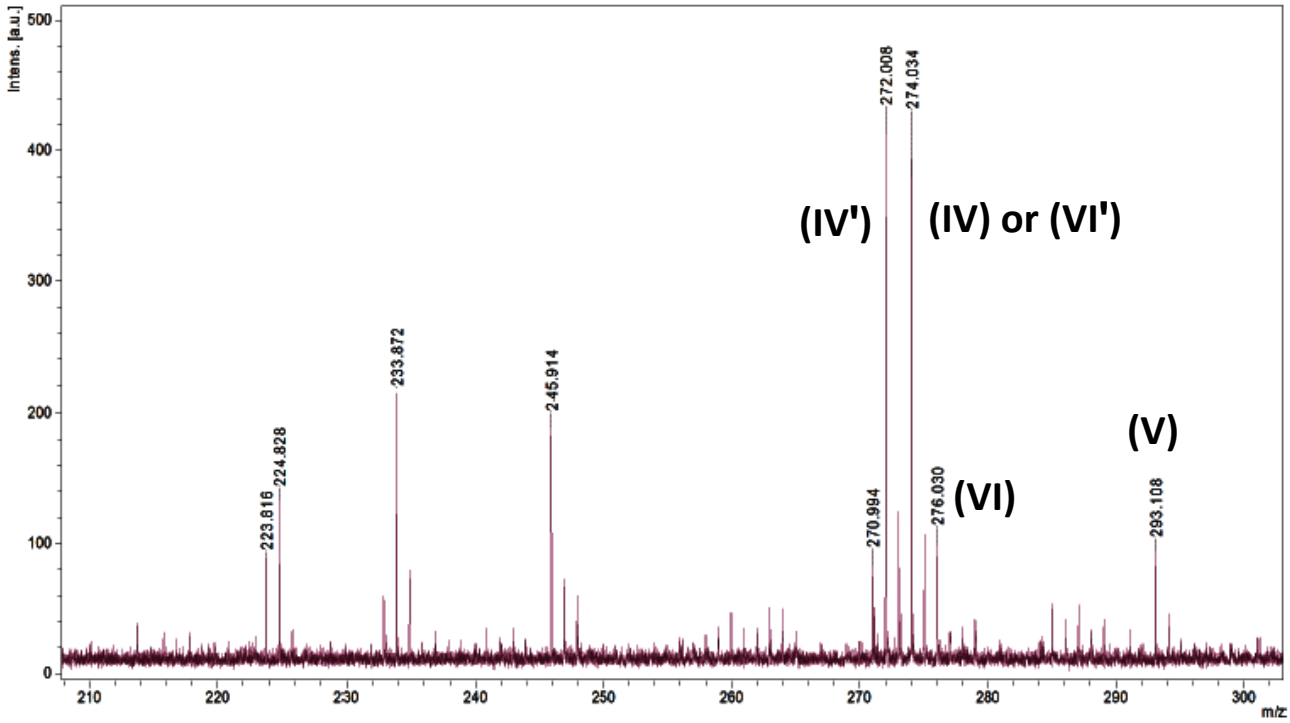


Figure S9: MALDI TOF mass spectra of serotonin-OPA (5HT-OPA) adducts.

m/z= 272.006 indicate formation of IV′(m/z_{cal} =272.09).

m/z= 274.034indicate formation of IV(m/z_{cal} =274.11).

m/z= 276.030 indicate formation of VI(m/z_{cal} = 276.13).

m/z= 293.106indicate formation ofV (m/z_{cal} =292.12).

4.2 ESI-MS spectra of the serotonin (5-HT) OPA adduct

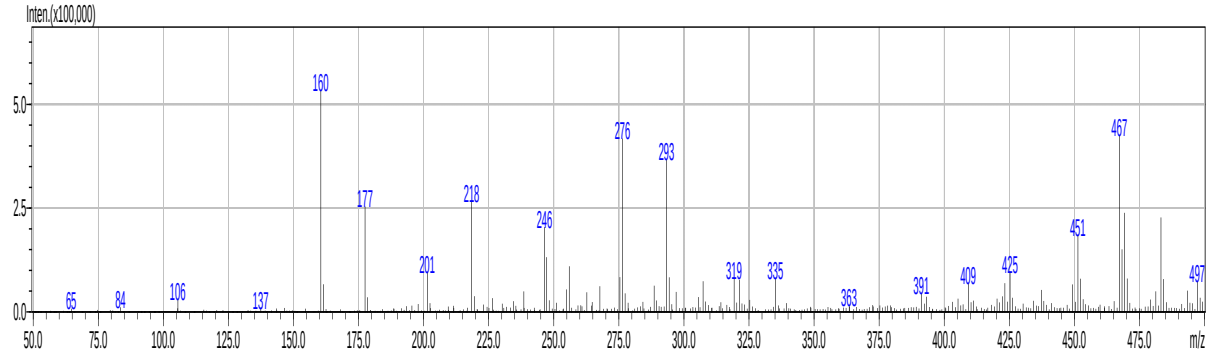


Figure S10: ESI Mass spectra of serotonin-OPA (5HT-OPA) adduct.

m/z= 276 indicate formation of VI (m/z_{cal} = 276.13)

m/z= 293 indicate formation of V(m/z_{cal} =292.12)

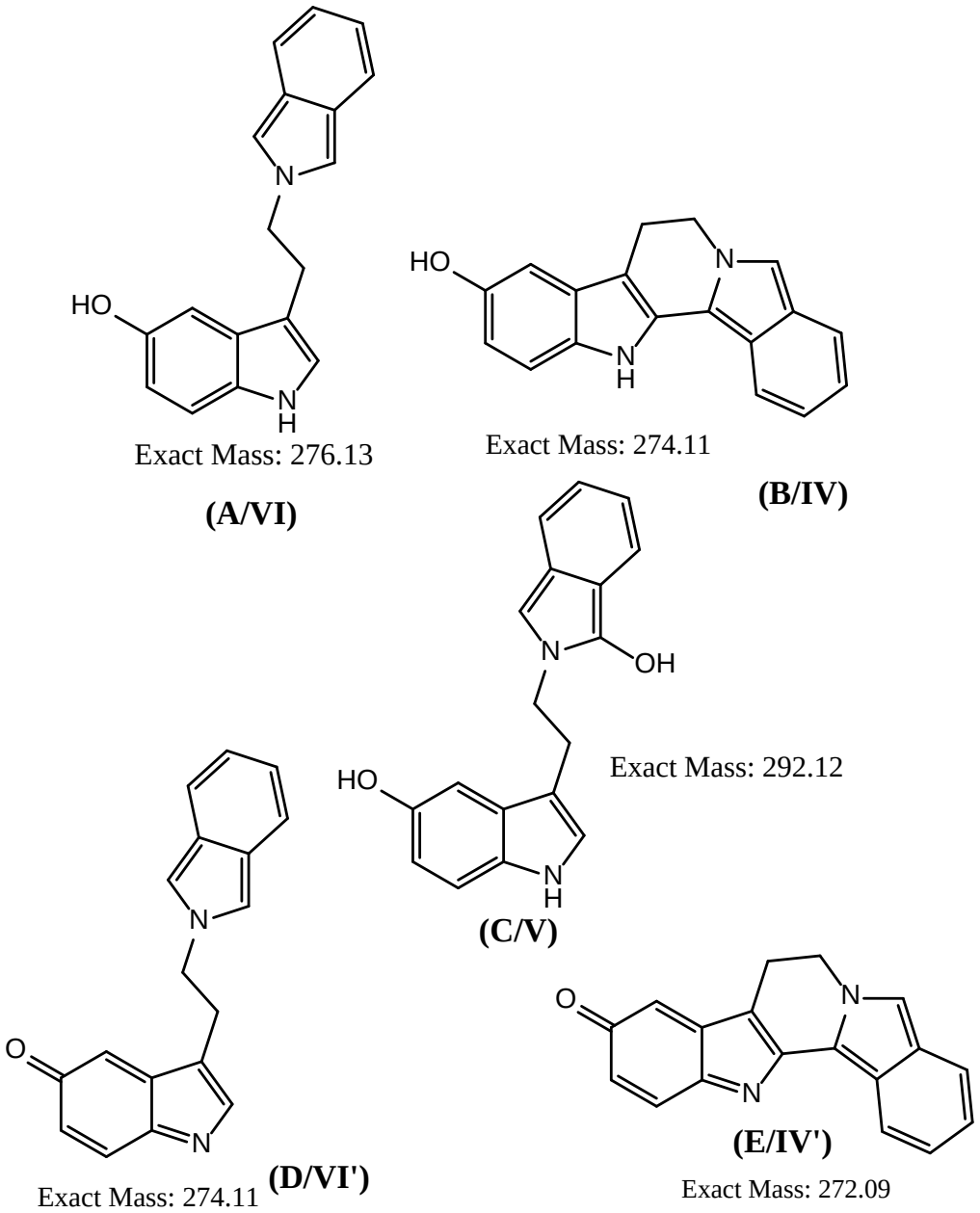


Figure S11: Probable serotonin-OPA (**5HT-OPA**) adducts and their calculated mass.

5. Fluorescence spectroscopy of DA-OPA and 5HT-OPA adduct: Fluorescence spectra are recorded using a Fluoromax equipped with a xenon lamp. Samples(DA-OPA and 5HT-OPA adduct) for emission measurements are contained in quartz cuvettes (1 mL volume). Excitation is provided at 450 nm with excitation and emission slit widths at 5/5 nm. All spectroscopic measurements are performed under two different conditions (in 10 mM PBS buffer, pH 7.4 & pH 5.5).

6. MN9D, RN46A and HEK cell culture. MN9D dopaminergic cells and HEK cellsof low passage numbers were cultured in DMEM media (Gibco, USA)supplemented with 10% FBS, 50 units/ml penicillin and 50 μg/mL streptomycin (Gibco, USA) at 37 °C under humidified air containing5% CO_2 in T-25 canted-neck flasks. For RN46A serotonergic cells, DMEM-F12 (1:1) (Gibco, USA) media was used instead of regular DMEM. Media was changed every 48hours.

7. Single photon imaging of cells. For cell imaging studies, all celltypes were grownon a poly-L -lysine-coated (0.1 mg/mL)homemade glass coverslip-bottomed Petri dishes. Cellimaging was performed using a confocal microscope (LSM 710,Carl Zeiss, Germany) with a 40X water immersion objective.Fluorescence images were recorded at an interval of 0.5 μm inthe longitudinal (Z) direction. Different cell types wereincubated with 100 μM of OPA in Thomson's buffer (TB, 146mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 0.4 mM KH_2PO_4, 0.3 mM Na_2HPO_4, 5 mM dextrose, and 20 mMNa-HEPES, pH adjusted to 7.4) for 30 min. Subsequent washeswere given with TB to remove the remaining OPA. The cellswere then imaged (excitationwavelength, 488nm).All the salt buffers were purchased from sigma.

8. Colocalization experiments (multiphoton imaging followed by single photon imaging). The RN46A serotonergic cells (without OPA treatment) were first imaged using multiphoton excitation to capture the intrinsic fluorescence of serotonin, followed by single photon excitation of OPA-serotonin adduct after OPA treatment and probed for co-localization.Briefly, on a custom modified confocal microscope (LSM710, Zeiss, Germany) an external self mode-locked Ti-Sapphire femtosecond laser (MaiTai, Spectra-Physics, CA, USA) is used for multiphoton excitation. Briefly, 740 nm light (from the tune-able range of 690-1040 nm of MaiTai) were routed to the scan box of the confocal microscope. The excitation laser (mildly diverging) was collimated with a 100cm convex lens. A combination of a half wave-plate and a polarizer was placed to control the excitation power. The custom modification allowed us to attach our detectors and detector housing to the non-descanned port of the LSM710 confocal microscope. The excitation was routed to the sample through a dichroic (MBS690+, Zeiss, Germany) in the confocal scan box to another dichroic (MBS690+, Zeiss, Germany or 720 nm short pass dichroic, Semrock, USA) placed right below the objective lens (40× water immersion Zeiss, Germany) at 45° angle. The fluorescence collected back by the same objective was separated from the excitation by the dichroic below the objective and sent to the non-descanned port of the microscope. The non-descanned port was custom modified to accommodate the external PMT and process the signal recorded by it. We used a near-UV sensitive PMT (P30A-01, Electron tubes, UK), directly coupled to the side exit port, for better detection of UV fluorophores. The

fluorescence was filtered through a 1 cm thick $CuSO_4$ solution to get rid of the residual excitation light. The other dichroic in the scan box is just for guiding the excitation light to the sample. For the single photon excitation, the protocol was followed as mentioned in the previous section.

9. Mass Spectrometry with OPA treated RN46A Cells

High-passage RN46A cells are cultured in DMEM-F12 (1:1) media supplemented with 10% FBS, 50 units/ml penicillin, and 50 µg/mL streptomycin at 37°C under humidified air containing 5% CO_2 in T-25 canted-neck flasks. Then the media is washed with Thomson's buffer and incubated with 100 µM OPA (2 ml) for 1 hour. Again the cells is washed thoroughly with Thomson's buffer, sonicated for 1 h to crush the cells and the soupe is collected by filtration for mass spectrometry. For control experiment we have followed same experiment without OPA.

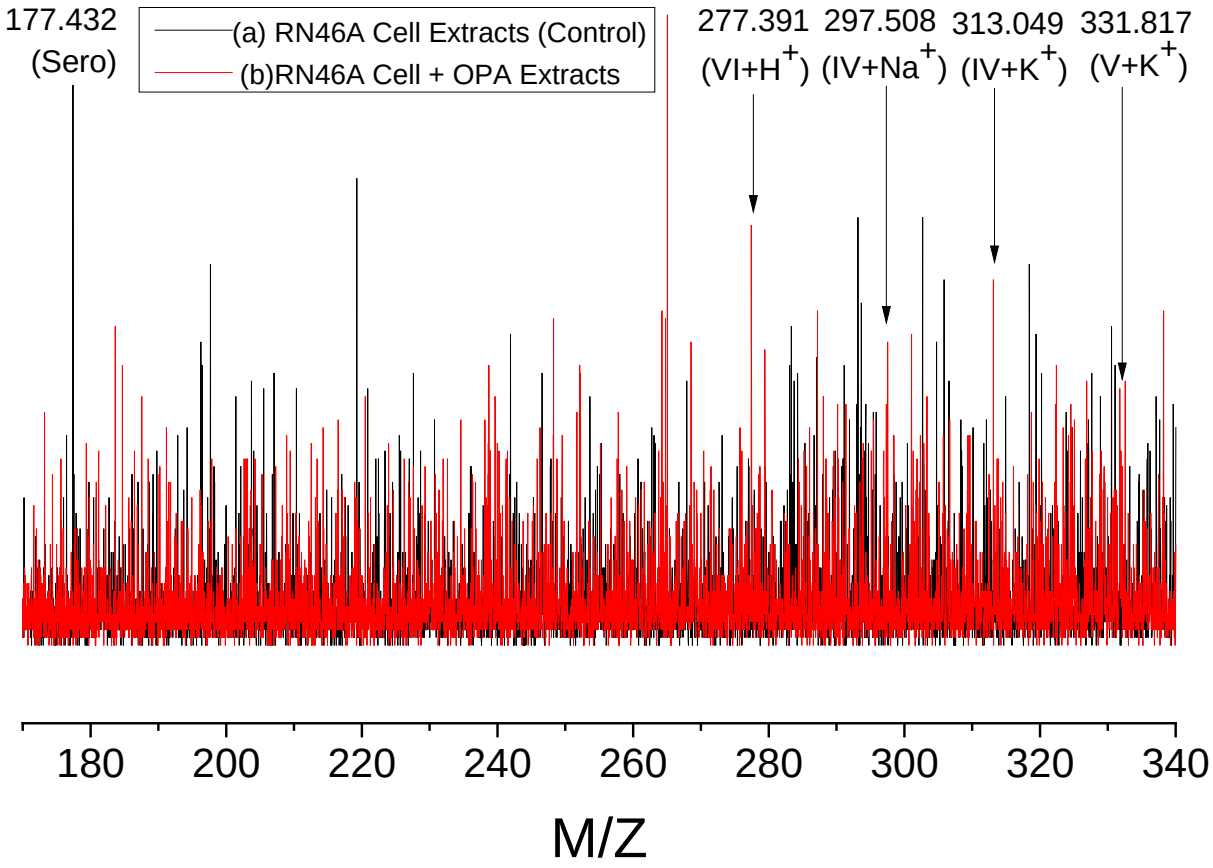


Figure S12: MALDI-TOF mass spectra of (a) RN46A cell + 100 µM OPA extract and (b) only RN46A cell extract.